

ASTHMA:- kinetics steady K3 immunized

RM134L:  $\frac{400}{2000} \times 8000 = 1600 + 6400$  first challenged.

EXHIBIT G

DAY 0 :- ~~2~~

BAL cell count (300ul). 2

C<sub>1</sub> :-  $2 \times 10^4 = 0.2 \times 10^5$  cells.

C<sub>2</sub> :-  $13 \times 10^4 = 1.3 \times 10^5$  cells.

A<sub>1</sub> :-  $9 \times 10^4 = 0.9 \times 10^5$  cells.

A<sub>2</sub> :-  $12 \times 10^4 = 1.2 \times 10^5$  cells.

B<sub>1</sub> :-  $11 \times 10^4 = 1.1 \times 10^5$  cells.

B<sub>2</sub> :-  $8 \times 10^4 = 0.8 \times 10^5$  cells.

C = control i.e. no Alum

A = immunized with Alum only

B<sub>1</sub> = " " Alum-OVA

9000

120000

time cell count:-

|        |  | Total / mice                   | <sup>Total volume</sup><br>in 10 mice / mice |
|--------|--|--------------------------------|--|
| 2 mice | C :- LN smpl = $728 \times 2 \times 10^4$              | $= 1.456 \times 10^7$ cells/ml | $\times 0.5/2 = 3.64 \times 10^6$            |
| 2 mice | (10ml) LUNG = $57.75 \times 10^4$                      | $= 2.86 \times 10^6$ cells/ml  | $\times 10/2 = 1.425 \times 10^7$            |
| "      | (10ml) Spleen = $448 \times 5 \times 10^4$             | $= 2.240 \times 10^7$ cells/ml | $\times 10/2 = 1.12 \times 10^8$             |
| 2 mice | A :- LN smpl = $629 \times 2 \times 10^4$              | $= 1.256 \times 10^7$ cells/ml | $\times 0.5/2 = 3.14 \times 10^6$            |
| "      | (10ml) LUNG = $71.15 \times 10^4$                      | $= 3.55 \times 10^6$ cells/ml  | $\times 10/2 = 1.775 \times 10^7$            |
| "      | (10ml) Spleen = $507 \times 5 \times 10^4$             | $= 2.535 \times 10^7$ cells/ml | $\times 10/2 = 1.27 \times 10^8$             |
| 2 mice | B <sub>1</sub> :- LN smpl = $849 \times 2 \times 10^4$ | $= 1.696 \times 10^7$ cells/ml | $\times 0.5/2 = 4.25 \times 10^6$            |
| "      | (10ml) LUNG = $59.45 \times 10^4$                      | $= 2.95 \times 10^6$ cells/ml  | $\times 10/2 = 1.475 \times 10^7$            |
| "      | (10ml) Spleen = $391.5 \times 10^4$                    | $= 1.955 \times 10^7$ cells/ml | $\times 10/2 = 9.775 \times 10^7$            |

1st 20K40L → 2nd. CD44 + 80p

DAY ①.

DAY ②.

RM1342 :- 12 mice  $\rightarrow$  6ml make up 7ml.

$$\text{stock} = 2\text{mg/ml} \quad \frac{400}{2000} \times 7000 = 1400 \quad (1500 + 5500)$$

$$\text{collagenase} = \frac{5\text{mg}}{1000} \times 3000 = 150\text{ml} \times 2 = 300\text{ml}$$

$$\text{DNAse} = \frac{0.1}{10} \times 3000 = 30\text{ml} \rightarrow 40\text{ml}$$

BAL cell count : (300ml).

A = Alen only

B = Alen - QVA.

C = Alen - QVA / RM1342

\* FACS:

$$* A_1 = 10 \times 10^4 = 1 \times 10^5$$

$$* A_2 = 6 \times 10^4 = 0.6 \times 10^5$$

$$* B_1 = 21 \times 10^4 = 2.1 \times 10^5$$

$$* B_2 = 33 \times 10^4 = 3.3 \times 10^5$$

$$* B_3 = 22 \times 10^4 = 2.2 \times 10^5$$

$$* B_4 = 10 \times 10^4 = 1 \times 10^5$$

$$* C_1 = 12 \times 10^4 = 1.2 \times 10^5$$

$$* C_2 = 8 \times 10^4 = 0.8 \times 10^5$$

$$* C_3 = 9 \times 10^4 = 0.9 \times 10^5$$

$$* C_4 = 4 \times 10^4 = 0.4 \times 10^5$$

Survival count:- Day 21

|            |   |   | total<br>volume          | total/mice                       |
|------------|---|---|--------------------------|----------------------------------|
| 2 mice/gp. | A | LN (1ml) = $238 \times 5 \times 10^4 =$       | $1.19 \times 10^7$ cells | $\times 1/2 = 5.95 \times 10^6$  |
| "          |   | LUNG (10ml) = $54 \times 5 \times 10^4 =$     | $2.70 \times 10^6$ cells | $\times 1/2 = 1.35 \times 10^6$  |
| "          |   | Spleen (10ml) = $169 \times 10 \times 10^4 =$ | $1.69 \times 10^7$ cells | $\times 1/2 = 8.45 \times 10^6$  |
| "          | B | LN (1ml) = $358 \times 5 \times 10^4 =$       | $1.79 \times 10^7$ cells | $\times 1/2 = 8.95 \times 10^6$  |
| "          |   | LUNG (10ml) = $83 \times 5 \times 10^4 =$     | $4.15 \times 10^6$ cells | $\times 1/2 = 2.075 \times 10^6$ |
| "          |   | Spleen (10ml) = $175 \times 10 \times 10^4 =$ | $1.75 \times 10^7$ cells | $\times 1/2 = 8.75 \times 10^6$  |
| "          | C | LN (1ml) = $216 \times 5 \times 10^4 =$       | $1.08 \times 10^7$ cells | $\times 1/2 = 5.40 \times 10^6$  |
| "          |   | LUNG (10ml) = $46 \times 5 \times 10^4 =$     | $2.30 \times 10^6$ cells | $\times 1/2 = 1.15 \times 10^6$  |
| "          |   | Spleen (10ml) = $203 \times 10 \times 10^4 =$ | $2.03 \times 10^7$ cells | $\times 1/2 = 1.015 \times 10^7$ |

FACS staining:- DX40L = 40 well 50  $\mu$ l/well. 2ml + 40  $\mu$ l of 90

~~CD11b~~ B220 } 1400  $\mu$ l + 25  $\mu$ l. + 40  $\mu$ l of 90  
~~B220~~ CD11b } 1400  $\mu$ l  
 CD11b } 1400  $\mu$ l.

DAY 2 :: K3 study.

$$\text{RMB4L} = \frac{500}{2000} \times 5000 = 1000 + 400$$

BAC cell count (300ul).

apexi LUNG

$$\text{PBS} \cdot * A_1 = 14 \times 10^4 = 1.4 \times 10^5$$

$$* * A_2 = 9 \times 10^4 = 0.9 \times 10^5$$

$$* B_1 = 70 \times 10^4 = 7.0 \times 10^5$$

$$* * B_2 = 261 \times 10^4 = 2.61 \times 10^6$$

$$* * B_3 = 59 \times 10^4 = 5.9 \times 10^5$$

$$* * B_4 = 356 \times 10^4 = 3.56 \times 10^6$$

$$* * C_1 = 7 \times 10^4 = 0.7 \times 10^5$$

$$* * C_2 = 8 \times 10^4 = 0.8 \times 10^5$$

$$\text{PBS} \cdot C_3 = 59 \times 10^4 = 0.59 \times 10^5$$

$$\text{PBS} \cdot * C_4 = 50 \times 10^4 = 5.0 \times 10^5$$

Tissue cell counts:

Total volume  
divided  
time

|   |                                    | total / 2 min                                  | total / min  |
|---|------------------------------------|--|--|
| A | LN (1ml) $\times \frac{1}{2}$      | $101 \times 4 \times 10^4 = 4.04 \times 10^6$  | $4.04 \times 10^6 \times 1/2 = 2.02 \times 10^6 \text{ cells}$ |
|   | LUNG (10ml) $\times \frac{1}{2}$   | $70 \times 5 \times 10^4 = 350 \times 10^4$    | $350 \times 10^4 \times 1/2 = 1.75 \times 10^7 \text{ cells}$  |
|   | Spleen (10ml) $\times \frac{1}{2}$ | $133 \times 10 \times 10^4 = 1.33 \times 10^7$ | $1.33 \times 10^7 \times 1/2 = 6.65 \times 10^6 \text{ cells}$ |

6

|   |               |  |                                  |
|---|---------------|--|----------------------------------|
| B | LN (1ml)      | $= 685 \times 4 \times 10^4 = 2.74 \times 10^7$  | $\times 1/2 = 1.37 \times 10^7$  |
|   | LUNG (10ml)   | $= 135 \times 5 \times 10^4 = 6.75 \times 10^6$  | $\times 1/2 = 3.375 \times 10^6$ |
|   | Spleen (10ml) | $= 191 \times 10 \times 10^4 = 1.91 \times 10^7$ | $\times 1/2 = 9.05 \times 10^6$  |

6

|   |               |  |                                 |
|---|---------------|--|---------------------------------|
| C | LN (1ml)      | $= 325 \times 4 \times 10^4 = 1.3 \times 10^7$   | $\times 1/2 = 6.5 \times 10^6$  |
|   | LUNG (10ml)   | $= 88 \times 5 \times 10^4 = 4.4 \times 10^6$    | $\times 1/2 = 2.2 \times 10^6$  |
|   | Spleen (10ml) | $= 234 \times 10 \times 10^4 = 2.34 \times 10^7$ | $\times 1/2 = 1.17 \times 10^7$ |

DAY (3)

W3 Study:-

$$\frac{1.117 \mu\text{L} \times 400}{2000} \times 2500 = \underline{500 \mu\text{L}}$$

80% cell count is - A (300  $\mu\text{L}$ ).

LFPS LUNG

|   |                |   |   |   |                           |
|---|----------------|---|---|---|---------------------------|
| * | A <sub>1</sub> | = | <del>14</del> $19 \times 2 \times 10^4$   | = | $3.8 \times 10^5$ cells.  |
| * | A <sub>2</sub> | = | <del>20</del> $20 \times 2 \times 10^4$   | = | $4.0 \times 10^5$ cells.  |
| * | B <sub>1</sub> | = | <del>295</del> $295 \times 2 \times 10^4$ | = | $5.90 \times 10^5$ cells. |
| * | B <sub>2</sub> | = | <del>30</del> $30 \times 2 \times 10^4$   | = | $6.0 \times 10^5$ cells.  |
| * | B <sub>3</sub> | = | $295 \times 2 \times 10^4$                | = | $5.90 \times 10^5$ cells. |
| * | B <sub>4</sub> | = | $200 \times 2 \times 10^4$                | = | $4.00 \times 10^5$ cells. |
| * | C <sub>1</sub> | = | $139 \times 2 \times 10^4$                | = | $2.78 \times 10^5$ cells. |
| * | C <sub>2</sub> | = | $68 \times 2 \times 10^4$                 | = | $1.36 \times 10^5$ cells. |
| * | C <sub>3</sub> | = | $8 \times 2 \times 10^4$                  | = | $0.6 \times 10^5$ cells.  |
| * | C <sub>4</sub> | = | $28 \times 2 \times 10^4$                 | = | $5.6 \times 10^5$ cells.  |

liver cell count:-

|   |                    |   | total / min                 | total / min  |
|---|--------------------|---|-----------------------------|--|
| A | LN (1ml) 125       | = | $151 \times 5 \times 10^4$  | = $7.55 \times 10^6 \times 1/2 = 3.78 \times 10^6$   |
|   | LUNG (10ml) 1:5    | = | $97 \times 5 \times 10^4$   | = $4.35 \times 10^6 \times 10/2 = 2.175 \times 10^7$ |
|   | Spleen (10ml) 1:10 | = | $99 \times 10 \times 10^4$  | = $9.90 \times 10^6 \times 10/2 = 4.95 \times 10^7$  |
| B | LN (1ml)           | = | $808 \times 5 \times 10^4$  | = $4.04 \times 10^7 \times 1/2 = 2.02 \times 10^7$   |
|   | LUNG (10ml)        | = | $158 \times 5 \times 10^4$  | = $7.90 \times 10^6 \times 10/2 = 3.95 \times 10^7$  |
|   | Spleen (10ml)      | = | $166 \times 10 \times 10^4$ | = $1.66 \times 10^7 \times 10/2 = 8.30 \times 10^7$  |
| C | LN (1ml)           | = | $239 \times 5 \times 10^4$  | = $1.195 \times 10^7 \times 1/2 = 5.98 \times 10^6$  |
|   | LUNG (10ml)        | = | $49 \times 5 \times 10^4$   | = $2.45 \times 10^6 \times 10/2 = 1.225 \times 10^7$ |
|   | Spleen (10ml)      | = | $274 \times 10 \times 10^4$ | = $2.74 \times 10^7 \times 10/2 = 1.37 \times 10^8$  |

# DAY 4

## BAL cell count (3 quadr.)

info. invr

- \* \*  $A_1 = 10 \times 10^4 = 1.0 \times 10^5$  cells.
- \* \*  $A_2 = 12 \times 10^4 = 1.2 \times 10^5$  cells
- \* \* \*  $B_1 = 576 \times 2 \times 10^4 = 1.152 \times 10^7$  cells.
- $B_2 = 30 \times 2 \times 10^4 = 6.0 \times 10^5$  cells.
- \* \*  $B_3 = 140 \times 5 \times 10^4 = 7.00 \times 10^6$  cells.
- \* \* \*  $B_4 = 166 \times 5 \times 10^4 = 8.30 \times 10^6$  cells.
- \* \* \*  $C_1 = 97 \times 2 \times 10^4 = 1.94 \times 10^6$  cells.
- \* \* \*  $C_2 = 108 \times 2 \times 10^4 = 2.16 \times 10^6$  cells.
- \*  $C_3 = 20 \times 2 \times 10^4 = 4.0 \times 10^5$  cells.
- \* \*  $C_4 = 5 \times 2 \times 10^4 = 1.0 \times 10^5$  cells.

## trium cell count

|   |  | Total / 2mm                      | total volume<br># mm | total                |
|---|--|----------------------------------|----------------------|----------------------|
| A | LN (1ml) $\downarrow$ 115 = $2.35 \times 5 \times 10^4$  | $= 1.175 \times 10^7 \times 1/2$ |                      | $= 5.98 \times 10^6$ |
|   | LUNG (1ml) $\downarrow$ 115 = $104 \times 5 \times 10^4$ | $= 5.20 \times 10^6 \times 10/2$ |                      | $= 2.60 \times 10^7$ |
|   | spleen (1ml) $\downarrow$ = $86 \times 10 \times 10^4$   | $= 8.60 \times 10^6 \times 10/2$ |                      | $= 4.30 \times 10^7$ |

|   |  |                                   |  |                       |
|---|--|-----------------------------------|--|-----------------------|
| B | LN (1ml) $\downarrow$ 115 = $437 \times 10 \times 10^4$  | $= 4.370 \times 10^7 \times 1/2$  |  | $= 2.185 \times 10^7$ |
|   | LUNG (1ml) $\downarrow$ 115 = $191 \times 5 \times 10^4$ | $= 9.55 \times 10^6 \times 10/2$  |  | $= 4.775 \times 10^7$ |
|   | spleen (1ml) $\downarrow$ = $135 \times 10 \times 10^4$  | $= 1.350 \times 10^7 \times 10/2$ |  | $= 6.750 \times 10^6$ |

|   |  |                                   |  |                       |
|---|--|-----------------------------------|--|-----------------------|
| C | LN (1ml) $\downarrow$ 115 = $302 \times 5 \times 10^4$   | $= 1.510 \times 10^7 \times 1/2$  |  | $= 7.55 \times 10^6$  |
|   | LUNG (1ml) $\downarrow$ 115 = $266 \times 5 \times 10^4$ | $= 4.05 \times 10^7 \times 10/2$  |  | $= 2.025 \times 10^7$ |
|   | spleen (1ml) $\downarrow$ = $106 \times 10 \times 10^4$  | $= 1.060 \times 10^7 \times 10/2$ |  | $= 5.300 \times 10^6$ |

1. A LN CD4/OK40 DAY 0.
2. B
3. C
4. A LUNG
5. B
6. C
7. A spleen.
8. B
9. C
10. A BALF.
11. B
12. C
13. A LN CD4/OK40 DAY 1
14. B
15. C
16. A LUNG
17. B
18. C
19. A spleen.
20. B
21. C
22. A BALF
23. B
24. C
25. A LN CD4/OK40 DAY 2
26. B
27. C
28. A LUNG.
29. B
30. C

30. A spleen CD4/OK40 DAY2

32. B

33. C

34. A BALF.

35. B

36. C

37. A LN CD4/OK40 DAY3

38. B

39. C

40. A LUNG

41. B

42. C

43. A spleen

44. B

45. C

46. A BALF.

47. B

48. C

49. A LN CD4/OK40 DAY4.

50. B

51. C

52. A LUNG

53. B

54. C

55. A spleen.

56. B

57. C

58. A BALF.

59. B

60. C

61.



61 A LN B220/0X40L DAY 0  
 62 B  
 63 C  
 64 A LUNC  
 65 B  
 66 C  
 67 A spleen  
 68 B  
 69 C  
 70 A B2A Fluid  
 71 B  
 72 C  
 73 A LN B220/0X40L DAY 1  
 74 B  
 75 C  
 76 A LUNC  
 77 B  
 78 C  
 79 A spleen  
 80 B  
 81 C  
 82 A B2A Fluid  
 83 B  
 84 C  
 85 A LN B220/0X40L DAY 2  
 86 B  
 87 C  
 88 A LUNC  
 89 B  
 90 C  
 91

91 A spleen B220/OK402 DAY 2.

92 B

93 C

94 A BALF

95 B

96 C

97 A LN B220/OK402 DAY 3

98 B

99 C

100 A LUNG

101 B

102 C

103 A spleen.

104 B

105 C

106 A BALF.

107 B

108 C

109 A LN B220/OK402 DAY 4.

110 B

111 C

112 A LUNG

113 B

114 C

115 A spleen.

116 B

117 C

118 A BALF.

119 B

120 C

121

121. A LN CD11b/OX40L DAY 0.

122. B

123. C

124. A LUNG

125. B

126. C

127. A Spleen

128. B

129. C

130. A BALF

131. B

132. C

133. A LN CD11b/OX40L DAY 1

134. B

135. C

136. A LUNG

137. B

138. C

139. A Spleen

140. B

141. C

142. A BALF.

143. B

144. C

145. A LN CD11b/OX40L DAY 2

146. B

147. C

148. A LUNG

149. B

150. C

151.

151. A spleen CD11b/ OX402 DAY2.

152. B

153. C

154. A BACF

155. B

156. C

157. A LN CD11b/ OX402 DAY3

158. B

159. C

160. A LUNG ~~CD11b/ OX~~

161. B

162. C

163. A spleen.

164. B

165. C

166. A BACF.

167. B

168. C

169. A LN CD11b/ OX402 DAY4

170. B

171. C

172. A LUNG

173. B

174. C

175. A spleen.

176. B

177. C

178. A BACF.

179. B

180. C

181.

181. A LN CD11C/OX402 DAY 0.

182. B

183. C

184. A LUNG

185. B

186. C

187. A Spleen

188. B

189. C

190. A BALF.

191. B

192. C

193. A LN CD11C/OX402 DAY 1

194. B

195. C

196. A LUNG

197. B

198. C

199. A Spleen

200. B

201. C

202. A BALF.

203. B

204. C

205. A LN CD11C/OX402 DAY 2.

206. B

207. C

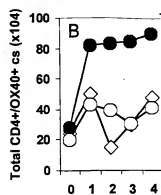
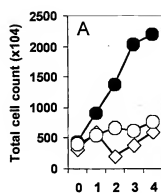
208. A LUNG.

209. B

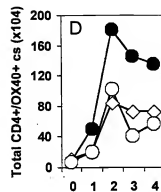
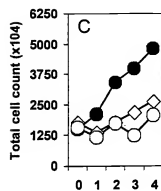
210. C

211.

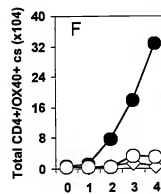
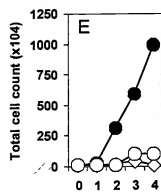
211. A spleen. CD11c / OX40L DAY 2.
212. B
213. C
214. A BALF.
215. B
216. C
217. A LN ~~CD11c~~ CD11c / OX40L DAY 3
218. B
219. C
220. A LUNC
221. B
222. C
223. A spleen.
224. B
225. C
226. A BALF.
227. B
228. C
229. A LN CD11c / OX40L DAY 4
230. B
231. C
232. A
233. B LADNC
234. C
235. A
236. B spleen.
237. C
238. A BALF.
239. B
240. C



LN

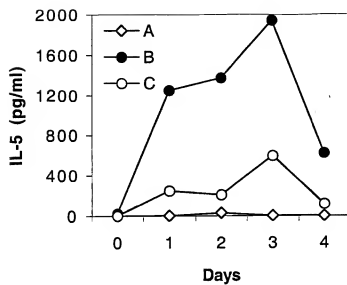


Lung



BALF

|      |    |       |        |      |       |
|------|----|-------|--------|------|-------|
| IL-4 | 0  | 1     | 2      | 3    | 4     |
| A    | 0  | 0     | 30     | 0    | 0     |
| B    | 22 | 1245  | 1370.5 | 1943 | 622.5 |
| C    | 0  | 248.5 | 209.5  | 596  | 116.5 |





IL-4  
A  
B  
C

|   |   |    |    |    |
|---|---|----|----|----|
| 0 | 1 | 2  | 3  | 4  |
| 0 | 0 | 0  | 0  | 0  |
| 0 | 0 | 82 | 60 | 52 |
| 0 | 0 | 14 | 16 | 15 |

